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Melatonin improves memory acquisition under stress independent of stress hormone release

Rimmele, U ; Spillmann, M ; Bärtschi, C ; Wolf, O T ; Weber, C S ; Ehlert, Ulrike ; Wirtz, P H

Abstract: **RATIONALE:** Animal studies suggest that the pineal hormone melatonin influences basal stress hormone levels and dampens hormone reactivity to stress. **OBJECTIVES:** We investigated whether melatonin also has a suppressive effect on stress-induced catecholamine and cortisol release in humans. As stress hormones affect memory processing, we further examined a possible accompanying modulation of memory function. **MATERIALS AND METHODS:** Fifty healthy young men received a single oral dose of either 3 mg melatonin ($n = 27$) or placebo medication ($n = 23$). One hour later, they were exposed to a standardized psychosocial laboratory stressor (Trier Social Stress Test). During stress, subjects encoded objects distributed in the test room, for which memory was assessed a day later ("memory encoding under stress"). Fifteen minutes following stress, memory retrieval for words learnt the day before was tested ("memory retrieval after stress"). Plasma epinephrine and norepinephrine levels, salivary free cortisol levels and psychological responses (attention, wakefulness) were repeatedly measured before and after stress exposure. **RESULTS:** Melatonin specifically enhanced recognition memory accuracy of objects encoded under stress ($p < 0.001$). In contrast, 15 min after stress, when cortisol levels were highest, retrieval of memories acquired the day before was not influenced by melatonin. Moreover, melatonin did not influence stress-induced elevation of catecholamine and cortisol levels which in turn did not correlate with the effects of melatonin on memory. **CONCLUSIONS:** The findings point to a primary action of melatonin on central nervous stimulus processing under conditions of stress and possibly on memory consolidation and exclude any substantial suppressive action of the substance on hormonal stress responses.

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Melatonin improves memory acquisition under stress independent of stress hormone release

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Abstract

Rationale Animal studies suggest that the pineal hormone melatonin influences basal stress hormone levels and dampens hormone reactivity to stress.

Objectives We investigated whether melatonin also has a suppressive effect on stress-induced catecholamine and cortisol release in humans. As stress hormones affect memory processing, we further examined a possible accompanying modulation of memory function.

Materials and methods Fifty healthy young men received a single oral dose of either 3 mg melatonin ($n=27$) or placebo medication ($n=23$). One hour later, they were exposed to a standardized psychosocial laboratory stressor (Trier Social Stress Test). During stress, subjects encoded objects distributed in the test room, for which memory was assessed a day later (“memory encoding under stress”).

Fifteen minutes following stress, memory retrieval for words learnt the day before was tested (“memory retrieval after stress”). Plasma epinephrine and norepinephrine levels, salivary free cortisol levels and psychological responses (attention, wakefulness) were repeatedly measured before and after stress exposure.

Results Melatonin specifically enhanced recognition memory accuracy of objects encoded under stress ($p<0.001$). In contrast, 15 min after stress, when cortisol levels were highest, retrieval of memories acquired the day before was not influenced by melatonin. Moreover, melatonin did not influence stress-induced elevation of catecholamine and cortisol levels which in turn did not correlate with the effects of melatonin on memory.

Conclusions The findings point to a primary action of melatonin on central nervous stimulus processing under conditions of stress and possibly on memory consolidation and exclude any substantial suppressive action of the substance on hormonal stress responses.

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Keywords Melatonin · Memory · Recognition · Stress ·
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Introduction

Acute stress activates the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis, leading to heightened levels of catecholamines (epinephrine and norepinephrine) and glucocorticoids (cortisol in primates, corticosterone in rodents; Mason 1968, Kirschbaum et al. 1993).

Besides the regulation of circadian rhythms and sleep (Brzezinski 1997; Cajochen et al. 2003), the pineal

hormone melatonin has been found to directly modulate catecholamine and cortisol levels. In rodents, exogenously administered melatonin decreases norepinephrine and epinephrine levels (Girouard et al. 2003; K-Laflamme et al. 1998; Viswanathan et al. 1986; Wang et al. 1999). Although not uniform, in young human subjects, placebo-controlled studies revealed decreased plasma levels of norepinephrine but not of epinephrine within 60–90 min after oral intake of 1 or 2 mg melatonin (Arangino et al. 1999; Cagnacci et al. 1998; Nishiyama et al. 2001). In contrast, effects of melatonin on cortisol/corticosterone levels are less consistent. In young humans, single or repeated administration of melatonin did not affect basal or pharmacologically stimulated cortisol secretion (Cagnacci et al. 1995; Forsling et al. 1999; Pawlikowski et al. 2002; Perras et al. 2005; Seabra et al. 2000; Waldhauser et al. 1987; Wright et al. 1986), whereas in rats and primates, the corresponding glucocorticoid measures were found to be decreased after melatonin (Konakchieva et al. 1997, 1998; Torres-Farfan et al. 2003). Notably, besides lowering basal corticosterone concentrations, in rats melatonin also attenuated corticosterone reactivity to an acute or chronic stressor (Konakchieva et al. 1997). Here we aimed to examine whether melatonin likewise attenuates cortisol as well as catecholamine reactivity to an acute stressor in humans.

Because the stress hormones of interest here, epinephrine, norepinephrine and cortisol, influence encoding, consolidation and retrieval of memories (Roozendaal et al. 2006), a second aim of our study was to examine whether a modulation of the stress response by melatonin would be accompanied by changes in memory processing.

In rodents and humans, increased levels of epinephrine and norepinephrine improve memory formation (Cahill and Alkire 2003; McGaugh and Roozendaal 2002; O'Carroll et al. 1999; Southwick et al. 2002), while blockade of the beta-adrenergic system inhibits memory formation (Cahill et al. 1994; van Stegeren et al. 1998). Furthermore, acute glucocorticoid administration or stress-induced endogenous glucocorticoid release specifically affects different memory phases in rodents and humans. As such, glucocorticoids dose-dependently enhance memory encoding and consolidation (Abercrombie et al. 2003; Beckner et al. 2006; de Kloet et al. 1999; Roozendaal 2000; Roozendaal et al. 2006) but distinctly impair memory retrieval (Buchanan et al. 2006; de Quervain et al. 1998, 2000; Domes et al. 2005). We expected that a dampening effect of melatonin on the hormonal stress response is accompanied by reduced encoding under stress (due to suppressed catecholamine and cortisol levels) but improved retrieval of memories (due to suppressed cortisol levels).

Materials and methods

Participants

The study is part of a larger project investigating effects of melatonin administration on psychobiological stress reactivity (Wirtz et al. 2008). The study sample consisted of 50 healthy, medication-free, non-smoking men (20–35 years). Subjects with any acute or chronic somatic or psychiatric disorders were excluded. Additional exclusion criteria, as obtained by subjects' self-report, included regular strenuous exercise, smoking, alcohol and illicit drug abuse, heart disease, elevated blood sugar and diabetes, elevated cholesterol, liver and renal diseases, chronic obstructive pulmonary disease, allergies and atopic diathesis, rheumatic diseases, current infectious diseases and insufficient knowledge of the German language. If the personal history was not conclusive, the subjects' primary-care physician was contacted for clarification. The Ethics Committee of the State of Zürich, Switzerland formally approved the research protocol. All participants provided written informed consent before participating and received 100 Swiss francs after participation.

Design and procedure

A placebo-controlled, single-blind, between-subjects design was used, with 27 men recruited for the melatonin group and 23 men in the placebo group. Raw data coding and analysis was conducted by an independent person blind to the drug condition. Participants in both groups took part in three experimental sessions taking place on three consecutive days (Fig. 1). They abstained from food and drinks (other than water) for 2 h prior to experimental sessions and from beverages with caffeine or alcohol and physical activity, starting on the evening 2 days before the first experimental session. Participants maintained a regular sleep–wake rhythm the three nights before and during the study, with sleep between 10–11.30 P.M. and 7–8.30 A.M.

Sessions started between 1 and 2 P.M. and, for each participant, always at the same time of day. On day 1, subjects were tested for normal cardiovascular functioning. After subjects had been sitting quietly for 20 min, heart rate and blood pressure were assessed by fully automated plethysmography (Omron 773, Mannheim, Germany). In the first session (day 1) subjects learnt a list of words that was used for later testing memory retrieval under conditions of stress during the second session (day 2). In the second session, hormonal responses to the Trier Social Stress Test (TSST) as well as memory under conditions of stress ("memory retrieval after stress") were tested after subjects had received either placebo or melatonin (3 mg, orally, "Melatonin Standard" purchased from PhysioLogics,

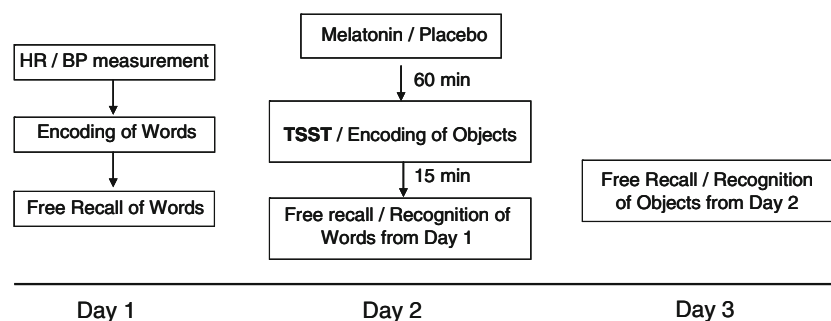


Fig. 1 Overview of the experimental sessions. Learning of words took place on day 1. On day 2, subjects were administered melatonin or placebo and underwent the psychosocial stress test (TSST). During

the TSST, subjects encoded memory for objects. After the TSST, subjects retrieved the words learnt on day 1. On day 3, retrieval of objects learnt during stress took place

Northglenn, CO, 80234 USA). Drugs were administered 60 min before the TSST, based on the known kinetics of melatonin after oral intake of 1–5 mg indicating that, at this time, blood levels of melatonin reach maximum values that are maintained for at least 2–4 h (Dollins et al. 1994; Waldhauser et al. 1984). The third session (day 3) served to test memory retrieval of items (objects distributed in the test room) that the subjects had encoded under conditions of stress (“memory encoding under stress”) on the second session on day 2.

Psychosocial stress procedure and hormone measurements

On the second session, 90 min after subjects had been prepared for blood sampling by insertion of an intravenous catheter and 60 min after oral drug intake, stress was induced by the Trier Social Stress Test. We applied the standard procedure of the TSST, which combines a short introduction phase followed by a 5-min preparation phase, a 5-min mock job interview and a 5-min mental arithmetic task in front of an unknown panel of one man and one woman. The TSST has repeatedly been found to induce profound endocrine and cardiovascular responses (Dickerson and Kemeny 2004; Kirschbaum et al. 1993).

Before and after the TSST, saliva and blood were sampled repeatedly to determine the hormonal stress response.

For assessment of free cortisol levels, saliva samples were collected immediately before (–1 min) and immediately after the TSST procedure (+1 min) as well as 10, 20, 30, 40, 50 and 60 min after completion of the TSST using Salivette collection devices (Sarstedt, Rommelsdorf, Germany). Samples were stored at –20°C until analysis with a commercially available competitive chemiluminescence immunoassay (LIA; IBL, Hamburg, Germany, sensitivity of 0.16 ng/ml). Intra- and inter-assay variability was less than 7.7% and 11.5%, respectively.

For assessment of plasma norepinephrine, epinephrine and melatonin levels, blood was sampled in ethylenedi-

amine tetraacetic acid-coated monovettes (Sarstedt, Numbrecht, Germany) immediately before (–1 min) and after the stressor (+1 min), as well as 10, 20 and 60 min after TSST completion. Blood was immediately centrifuged for 10 min at $2,000 \times g$ at 4°C; and plasma was stored at –80°C until analysis. Epinephrine and norepinephrine levels were determined by means of high-performance liquid chromatography (HPLC) and electrochemical detection after liquid–liquid extraction (Ehrenreich et al. 1997). Melatonin was extracted with methylene chloride and analysed with a reversed-phase HPLC with fluorescence detection (Vieira et al. 1992). The lower limit of detection (LOD) for the melatonin concentration was 2.5 pg/mL. Samples with a melatonin concentration below the LOD were set at half the LOD (i.e. 1.25 pg/ml) following previous recommendation for a skewed data set (Hornung and Reed 1990). Inter- and intra-assay coefficients of variation were <10%.

Testing of memory encoding under stress and memory retrieval after stress

Memory encoding under stress To examine the influence of melatonin on memory encoding under stress on day 2, 22 objects (e.g. a telephone, a glass, a bottle and a rubbish-bin) were placed in the TSST room. All objects were put in the subjects’ field of view. It was assured that subjects perceived all relevant objects during the TSST by instructing them to look around for a minute and to comment on the set-up of the room at the transition from the speech to the mental arithmetic part of the TSST. On day 3, free recall and recognition of the objects were tested. For recognition memory, photos of the 22 objects were presented with 44 similar objects as distractors.

Memory retrieval after stress For assessment of the influence of melatonin on memory retrieval under heightened cortisol levels, on day 1 (1 day prior to stress exposure), subjects were instructed to memorize 30 words

(10 positive, 10 neutral and 10 negative), which were presented in randomized order for 5 s each (Kuhlmann et al. 2005b). Immediately following the presentation, free recall of the words was tested. On day 2, a surprise delayed free recall test and recognition test of the words learnt the day before were employed 15 min after cessation of the TSST, when cortisol levels peak. For the recognition test, 60 distractor words matched to the target words for valence (20 positive, 20 neutral and 20 negative) were included (Domes et al. 2004). To account for possible within- and between-subject variance in memory, free recall performance after stress was expressed as the percentage of words remembered in relation to the words remembered immediately after learning on the day before (Kuhlmann et al. 2005a, b).

Measures of recognition memory were determined according to the two-threshold model (Snodgrass and Corwin 1988), including hit rate (HR = proportion of old objects classified as “old”), false alarm rate (FAR = proportion of new objects classified as “old”), the memory accuracy measure Pr [= HR–FAR] and the response bias measure Br [=FAR/(1–Pr)].

Assessment of attention, mood and anxiety

Influence of melatonin on attention, concentration, wakefulness and state anxiety were assessed before and after the stressor using the d2 letter cancellation test of attention (–65 min; +35 min; Brickenkamp 1994), the wakefulness scale of the Multidimensional Mood Questionnaire (MDBF; –80 min, –20 min, +20 min; Steyerberg et al. 2001) and the state scale of the State-Trait-Anxiety-Inventory (STAI; –85 min, –20 min, +5 min; Spielberger et al. 1970). Baseline measures for attention, wakefulness and state anxiety were obtained before melatonin or placebo administration. All questionnaires have been broadly used and shown satisfactory internal consistency and validity.

Statistical analyses

SPSS (version 14.0) software packages (SPSS, Chicago, IL, USA) was used for data analysis. Data are presented as mean \pm SEM. All tests were two-sided, and the level of significance was set at $p < 0.05$. Missing data were excluded listwise.

To determine changes in hormone levels and psychological measures, two-way analyses of variance (ANOVAs) with repeated measures were calculated with group (melatonin vs. placebo) as between-subject factor and time as within-subject factor (repeated factor: 2 for attention, 3 for wakefulness and state anxiety, 5 for epinephrine and norepinephrine, 8 for cortisol). To account for effects of repeated measurements, we applied Huynh–Feldt corrections for repeated measures. We present corrected p values but provide the original degrees of freedom without Huynh–Feldt correction. Baseline hormonal levels as well as differences in memory performance between the two groups were examined by one-way ANOVAs. For stress hormones, areas under the total response curve with respect to ground (AUCg) were calculated with the trapezoid formula (Pruessner et al. 2003). In order to assess whether the stress hormones modulated the influence of melatonin on memory, respective hormone AUCg were introduced as covariates in the ANOVA.

Results

Group characteristics and melatonin levels

Table 1 provides subjects' characteristics for the two groups of men treated with 3 mg melatonin ($n=27$) and placebo ($n=23$). There were no differences in age, body mass index, heart rate and blood pressure (all $p > 0.11$). Also, attention performance on the d2 test, state anxiety and wakefulness

Table 1 Group characteristics of melatonin and placebo group

	Melatonin (3 mg; $N=27$)	Placebo ($N=23$)	p
Age (years)	24.41 \pm 0.70	25.70 \pm 0.93	0.267
Body mass index (kg/m ²)	23.01 \pm 0.51	23.17 \pm 0.50	0.826
Heart rate (day 1; beats/min)	70.48 \pm 2.23	64.95 \pm 2.57	0.110
MAP (day 1; mmHg)	94.65 \pm 1.65	93.98 \pm 2.10	0.800
Attention (d2; –65 min; score)	504.85 \pm 13.41	486.39 \pm 15.49	0.370
Wakefulness (MDBF; –80 min; score)	16.08 \pm 0.54	15.22 \pm 0.55	0.274
State anxiety (STAI; –85 min; score)	36.88 \pm 1.90	35.36 \pm 1.71	0.560
Hit rate immediate recall (day 1; score)	0.62 \pm 0.03	0.63 \pm 0.02	0.730
False alarm rate immediate recall (day 1; score)	0.59 \pm 0.15	0.52 \pm 0.17	0.755

Values given are mean \pm SEM

MAP mean arterial blood pressure, calculated as 2/3 diastolic blood pressure + 1/3 systolic blood pressure; hit rate proportion of old objects classified as “old”; false alarm rate proportion of new objects classified as “old”; MDBF Multidimensional Mood Questionnaire

before drug intake on day 2 as well as immediate free recall of words (measured on day 1) were comparable between groups (melatonin: hits 18.59 ± 0.93 , false alarms 0.59 ± 0.15 ; placebo: hits 19.00 ± 0.65 , false alarms 0.52 ± 0.17 ; all p 's > 0.27). Measurement of melatonin in plasma samples 1 min prior to TSST exposure (day 2) confirmed higher plasma melatonin concentrations 60 min after substance intake in the melatonin group (421.8 ± 113.9 pg/ml, vs. 2.3 ± 0.4 pg/ml in the placebo group $F(1,48)=12.00$, $p<0.001$).

Stress hormone levels at baseline and stress responses

At baseline Resting levels of cortisol ($p=0.57$), norepinephrine ($p=0.22$) and epinephrine ($p=0.41$) did not differ between groups.

Stress responses Results on catecholamine stress reactivity have been previously reported (Wirtz et al. 2008). The TSST induced strong increases in cortisol ($F(7,301)=29.1$, $p<0.001$), norepinephrine ($F(4,176)=66.9$, $p<0.001$) and epinephrine levels ($F(4,168)=16.5$, $p<0.001$; Fig. 2). Compared with placebo after melatonin, epinephrine levels were, on average, slightly higher, and norepinephrine levels were, on average, slightly lower. However, these differences did not reach any significance. The groups did neither significantly differ before and after stress (main effect of group) nor in response to stress (interaction group-by-time) in cortisol (p 's > 0.47), norepinephrine (p 's > 0.18), and epinephrine (p 's > 0.14).

Effects of melatonin on memory encoding under stress and memory retrieval after stress

Memory encoding under stress Recognition of objects that were encoded during TSST performance (day 2) was measured on day 3. Recognition testing revealed large differences between groups (Fig. 3 summarizes results for hit rate, false alarm rate, memory accuracy and response bias). Subjects in the melatonin group compared to the placebo group showed distinctly enhanced memory accuracy (Pr; melatonin 0.32 ± 0.02 vs. placebo 0.22 ± 0.04 , $F(1,49)=5.44$, $p<0.05$) for objects encoded under stress exposure. An overall higher hit rate (melatonin 0.50 ± 0.02 vs. placebo 0.38 ± 0.02 , $F(1,49)=22.15$, $p<0.001$) contributed to this effect. False alarm rate ($p=0.58$) and response bias (Br; $p=0.07$) did not significantly differ between the groups. Free recall of objects did not reveal any significant difference between the melatonin (hits 6.52 ± 0.35 , false alarms 0.48 ± 0.14) and placebo (hits 6.17 ± 0.33 , false alarms 0.48 ± 0.17) groups (all p 's > 0.48).

Memory retrieval after stress As to retrieval function under conditions of stress, melatonin did not influence free recall

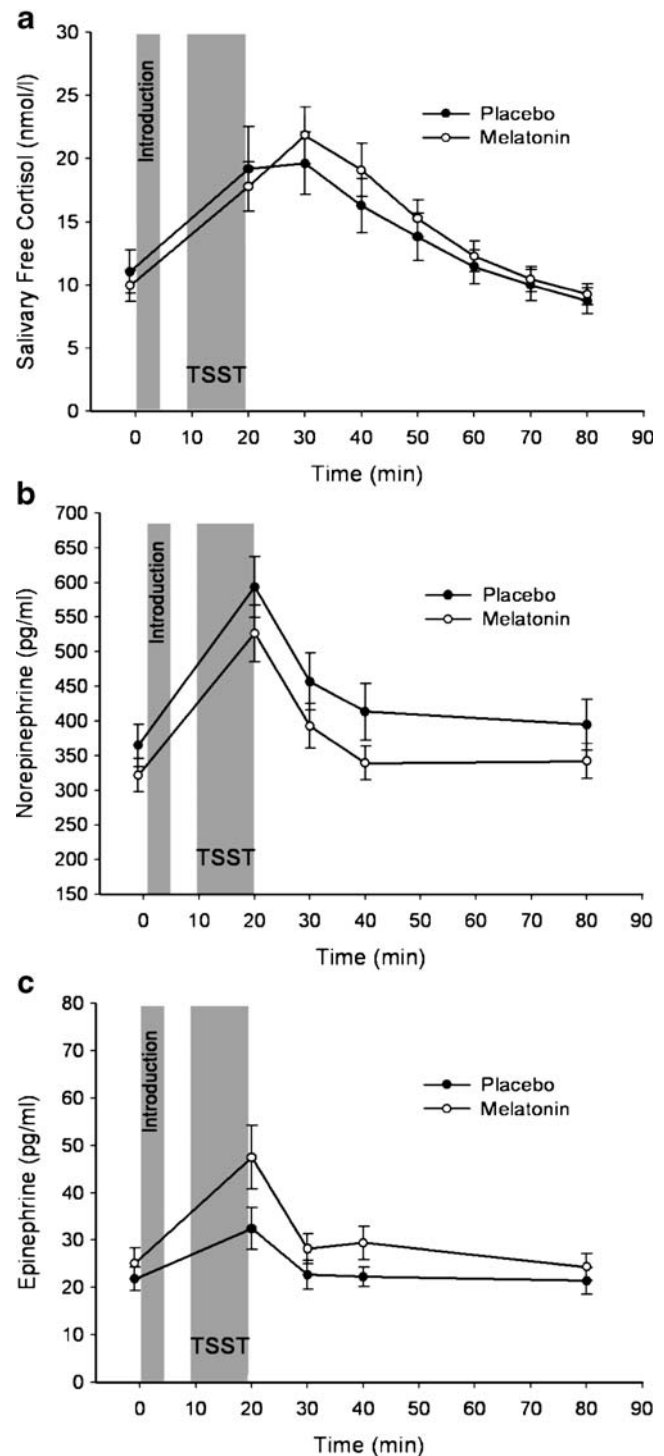
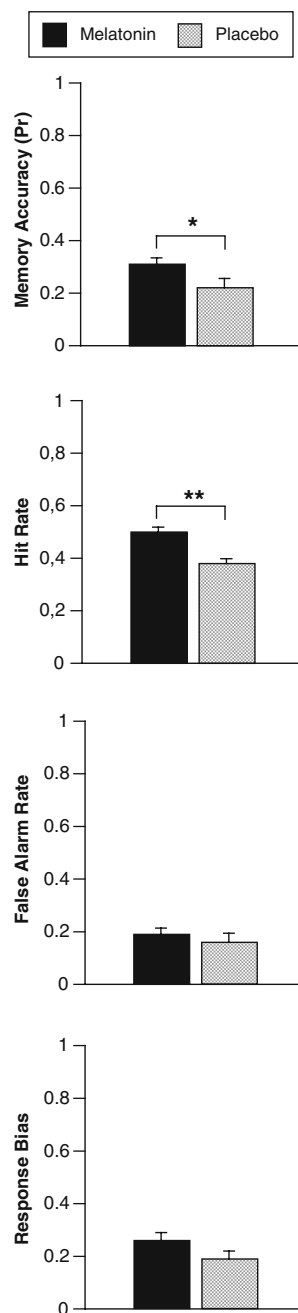


Fig. 2 a, b Values are means \pm SEM. Across all subjects, the Trier Social Stress Test (large shaded area) elicited a significant response in stress hormone levels (a–c). The TSST combines a short introduction phase followed by a 5-min preparation phase, a 5-min mock job interview and a 5-min mental arithmetic task in front of an unknown panel of one man and one woman. The melatonin and the placebo group did not differ in hormonal reactivity to the TSST (all p 's > 0.14)

Fig. 3 Values are means \pm SEM. Melatonin compared to placebo improved recognition memory for the objects encoded during the Trier Social Stress Test stress. Melatonin enhanced memory accuracy (hit rate minus false alarm rate). This effect was due to a higher hit rate for object recognition in the melatonin group. False alarm rate and response biases did not differ between groups



of words (15 min after stress on day 2) learnt on day 1 (main effect of treatment, $p > 0.49$; melatonin: hits 13.00 ± 0.87 , false alarms 1.22 ± 0.29 ; placebo: hits 12.70 ± 0.72 , false alarms 1.00 ± 0.27). Similarly, there was no influence of melatonin on the free recall of words depending on the valence (positive, neutral, negative) of the words ($p > 0.99$, for valence by treatment interaction; *positive words*: melatonin: hits 4.33 ± 0.31 ; placebo: hits 4.00 ± 0.31 ; *neutral words*: melatonin: hits 4.63 ± 0.42 , placebo: hits 4.48 ± 0.38 ; *negative words*: melatonin: hits 4.04 ± 0.38 ; placebo: hits 4.22 ± 0.36). Melatonin compared to placebo also had no influence on recognition measures of the words (melatonin:

hits 21.48 ± 1.39 , false alarms 4.04 ± 0.61 ; placebo: hits 23.43 ± 1.26 , false alarms 3.61 ± 0.54), regardless of valence (all p 's > 0.22 ; *positive words*: melatonin: hits 7.72 ± 0.32 ; placebo: hits 8.27 ± 0.40 ; *neutral words*: melatonin: hits 8.04 ± 0.38 , placebo: hits 8.45 ± 0.29 ; *negative words*: melatonin: hits 7.44 ± 0.30 ; placebo: hits 7.91 ± 0.29).

Influence of stress hormones on melatonin memory effects

Since previous studies indicated strong influences of cortisol and catecholamines on encoding and retrieval of memories (de Quervain et al. 2000; McGaugh and Roozendaal 2002), in supplementary analyses, we controlled for the stress response of each of these hormones by entering the respective AUCg as covariate into memory ANOVAs. None of these analyses of covariance did reveal any significance for any hormonal covariate (all p 's > 0.25) but confirmed the treatment effects reported above for the original analyses.

Attention, wakefulness, mood and anxiety

Attention significantly increased from before stress (-65 min; melatonin 504.85 ± 13.41 , placebo 486.39 ± 15.49) to the time after stress ($+35$ min; melatonin 538.93 ± 12.10 , placebo 525.57 ± 15.02 ; $F(1,48) = 147.8$, $p < 0.001$) but did not differ between groups (all p 's > 0.4). Wakefulness significantly decreased throughout the experiment (time effect: $F(2,94) = 14.36$, $p < 0.001$), with a stronger decrease in the melatonin group (group by time effect: $F(2,94) = 5.3$, $p < 0.01$; -80 min: melatonin 16.08 ± 0.54 , placebo 15.22 ± 0.55 ; -20 min: melatonin 13.26 ± 0.70 , placebo 14.3 ± 0.64 ; $+20$ min: melatonin 13.19 ± 0.65 , placebo 14.65 ± 0.68). It is worthy to note that the decrease in wakefulness as well as the increase in attention entered as covariate did not significantly change the reported melatonin effects on memory.

State anxiety significantly increased in both groups during anticipation of the psychosocial stressor (time effect: $F(2,86) = 42.38$, $p < 0.001$), but groups did not differ in their state anxiety response to the psychosocial stressor ($p > 0.69$).

Discussion

This is the first study to assess whether melatonin modulates hormonal stress reactivity in humans and whether this modulation would be accompanied by changes in memory processing. Oral administration of 3 mg melatonin did not substantially alter cortisol, epinephrine and norepinephrine responses to a psychosocial stressor.

However, melatonin invoked distinct changes in memory function. Going beyond previous studies examining effects of melatonin on encoding/consolidation and retrieval processes in combination, here we aimed at clearly dissociating effects of melatonin on these two types of memory processes. The central finding of our study is that melatonin specifically improved memory for objects that were presented together with the stressor, as indicated by enhanced accuracy and hit rate at a recognition test for these objects a day later. In contrast, 15 min after stress, when cortisol levels were highly elevated, memory retrieval of words learnt a day before remained unaffected by melatonin. Although we cannot demonstrate a cortisol-induced impairment of memory retrieval in both groups due to an absent non-stressed control group (Kuhlmann et al. 2005b, Buchanan et al. 2006; Domes et al. 2004), stress hormonal responses did not influence melatonin-modulated effects on retrieval. The findings point to a primary action of melatonin on central nervous stimulus processing under conditions of stress and exclude any substantial suppressive action of the substance on hormonal stress responses.

Notably, the melatonin-induced improvement of memory was revealed only in a recognition test but not in free recall testing. Recognition is a most direct test of the strength of an association in memory, whereas free recall, in contrast to recognition memory, is characterized by the virtual absence of external memory cues at retrieval and thus is an indicator of how a memory is cross-linked to other associated memories that could facilitate its accessibility (Lockhart 2000; Tulving 1970). On this background, the selective improvement of recognition performance strongly suggests that melatonin enhances associative strength, i.e. the signal-to-noise ratio at stimulus encoding under stress and possibly during consolidation.

The present results of melatonin effects on memory are difficult to integrate into results from the few previous studies investigating effects of melatonin on memory. First, most of these studies did not separately assess encoding and retrieval functions. Second, none of these studies tested memory function during stress, which per se affects memory encoding and retrieval (de Quervain et al. 1998; Elzinga et al. 2005; Kuhlmann et al. 2005b; Leeuwenberg et al. 1992). Concurring with the present observations, in adult male rats, melatonin facilitated social recognition (Argyriou et al. 1998), and in humans, several studies failed to reveal effects of melatonin on memory using free recall procedures (Rutledge et al. 2004; Waldhauser et al. 1984; Wesensten et al. 2005). However, Lieberman et al. (1984) found no effect of 240 mg melatonin on both free recall as well as recognition memory of words. In that study, both encoding and retrieval were measured at times of enhanced melatonin concentration, and the melatonin dose was distinctly higher, which may account for the divergent

findings. However, even with the 3-mg dose of the present study, plasma levels exceeding the normal physiological range were reached, making a saturation of receptors likely. Interestingly, in a recent study (Gorfine et al. 2007), subjects given 2 mg melatonin before a 2-h retention interval between encoding and retrieval showed, similarly to subjects who napped during the retention interval, higher activation in the left parahippocampus at successful retrieval of word pairs. This suggests that, in addition to the effect of encoding, melatonin can exert a sleep-like enhancing effect on consolidation of memories (Gorfine et al. 2007).

Consistent with numerous previous studies (Deacon and Arendt 1995; Dollins et al. 1994; Nickelsen et al. 1989; Rutledge et al. 2004; Waldhauser et al. 1984; Wright et al. 1986), melatonin compared to placebo decreased wakefulness. As this effect was not in relation to the memory effects of melatonin, wakefulness can be ruled out as a possible confound. Notably, the decrease in wakefulness also was not linked to changes in measures of attention and concentration. Performance on the d2 concentration test was closely comparable between the two treatment groups before and after the stressor. Indeed, the stressor caused a distinct increase in attention and concentration in both groups that possibly overrode any effect of melatonin in the opposite direction. In any case, the lack of differences in attention and concentration safely excludes that improved memory formation for objects encoded during stress was due to a non-specific attention function after melatonin.

Melatonin did not influence basal levels of cortisol, epinephrine and norepinephrine, which is in accordance with the majority of previous findings in healthy humans (Arangino et al. 1999; Cagnacci et al. 1995, 1998; Forsling et al. 1999; Nishiyama et al. 2001; Pawlikowski et al. 2002; Seabra et al. 2000; Waldhauser et al. 1987; Wright et al. 1986). Furthermore, melatonin did not affect hormonal reactivity to stress. This finding is consistent with studies that failed to reveal dampening effects of melatonin in men or rats on the glucocorticoid response to an emotional stressor or hypoglycaemia-induced HPA activation within 90 min after exposure to these stimuli (Konakchieva et al. 1998; Perras et al. 2005). While we did not find changes in basal norepinephrine levels 60 min after melatonin intake, other investigators observed lower norepinephrine levels in melatonin-treated young men when measured in a supine position but not in an upright position (Arangino et al. 1999; Nishiyama et al. 2001). Indeed, a particular sensitivity of orthostatic sympathetic regulation to the effects of melatonin is suggested as decreased norepinephrine levels in melatonin-treated subjects normalized after standing up for at least 15 min (Arangino et al. 1999; Nishiyama et al. 2001). Since our subjects were sitting during evaluation of baseline conditions, differences in body position may account for the observed discrepancy.

The neurophysiological mechanisms mediating the enhancing effect of melatonin on memory formation are elusive. Since no melatonin-induced changes in stress hormone levels were observed, our findings are unlikely to be mediated by cortisol, epinephrine or norepinephrine. We offer two explanations potentially underlying the effect of melatonin on memory encoding. First, melatonin binding sites exist in various brain structures involved in memory function such as the hippocampus and prefrontal cortex (Brzezinski 1997; Ekmekcioglu 2006; Mazzucchelli et al. 1996; Savaskan et al. 2001, 2005). Under non-stress conditions, melatonin reduced rather than improved induction of long-term potentiation (LTP) in hippocampal slices from mice, i.e. a synaptic plastic process believed to underlie the encoding of novels stimuli (Ozcan et al. 2006; Wang et al. 2005). Moreover, a recent study in zebrafish describes a suppressive effect of administration of a very high melatonin dose on nighttime memory formation under non-stress conditions (Rawashdeh et al. 2007). However, it is unknown whether these animal findings apply to daytime memory formation under resting conditions in humans. It is presently not known but conceivable that stress conditions as investigated in the present study promote the release of neuro-modulators (e.g. norepinephrine) that are able to shift the effect of melatonin on LTP induction into an opposite direction.

Second, considering that melatonin is a potent sleep-inducing factor (Wright et al. 1986) and sleep is known to enhance consolidation of hippocampus-dependent memories (Jern et al. 1991; Rasch et al. 2007), it is possible that ‘sleep-like’ melatonin effects on consolidation in the aftermath of encoding added to its effects on encoding. Indeed, since melatonin in the used dose is known to degrade to baseline levels in 4 to 8 h after oral ingestion (Brzezinski 1997), it is likely that melatonin also influenced early processes of memory consolidation in our study. However, this rationale implies a similar effect of melatonin also under basal resting conditions not investigated here, which limits the interpretation of our data.

In summary, our data indicate that melatonin rather than dampening hormonal responses to a social stressor exerts a genuinely central nervous effect by enhancing encoding of objects under stress and possibly memory consolidation. Strength and specificity of this effect call for investigating whether this effect emerges selectively during stress conditions or likewise during rest.

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